National Research Council Canada | Conseil national de recherches Canada

Canada

Reprinted from

Canadian Journal of Forest Research

Réimpression du

Journal canadien de recherche forestière

Effect of fertilization on free amino acid concentrations in black spruce and jack pine containerized seedlings

Y. T. Kim, C. Glerum, J. Stoddart, and S. J. Colombo

Volume 17 • Number 1 • 1987

Pages 27–30

Printed in Canada / Imprimé au Canada

Effect of fertilization on free amino acid concentrations in black spruce and jack pine containerized seedlings

Y. T. KIM, C. GLERUM, J. STODDART, AND S. J. COLOMBO
Ministry of Natural Resources, Ontario Tree Improvement and Forest Biomass Institute, Maple, Ont., Canada L0J 1E0

Received May 8, 1986

Accepted September 8, 1986

KIM, Y. T., C. GLERUM, J. STODDART, and S. J. COLOMBO. 1987. Effect of fertilization on free amino acid concentrations in black spruce and jack pine containerized seedlings. Can. J. For. Res. **17**: 27–30.

Greenhouse-grown black spruce and jack pine container seedlings were fertilized weekly with a 20–8–20 fertilizer at four concentrations during the fall. Seedlings were sampled when 23 weeks old towards the end of the greenhouse cultural period to determine the effect of fertilization on the free amino acid concentrations. All amino acids, except tryptophan, showed significant increases in concentration with higher levels of fertilizer; the concentration of tryptophan decreased with increasing fertilizer concentration. Amino acids with the highest concentrations in black spruce were arginine, glutamic acid, and proline, while in jack pine, besides these three, aspartic acid and glutamine were also found in high concentrations. Black spruce had significantly higher amino acid concentrations than jack pine. The concentrations of certain free amino acids may be more sensitive indicators of seedling nitrogen status than total foliar nitrogen.

KIM, Y. T., C. GLERUM, J. STODDART et S. J. COLOMBO. 1987. Effect of fertilization on free amino acid concentrations in black spruce and jack pine containerized seedlings. Can. J. For. Res. **17**: 27–30.

Des semis d'épinette noire et de pin gris en contentants, cultivés en serre, furent fertilisés chaque semaine durant l'automne avec un engrais 20–8–20, à quatre concentrations. Les semis furent échantillonnés à l'âge de 23 semaines, vers la fin de la période culturale en serre, pour déterminer l'effet de la fertilisation sur les teneurs en acides aminés libres. À l'exception du tryptophane, la concentration de tous les acides aminés a augmenté significativement avec l'augmentation des niveaux d'engrais. Les acides aminés dont la concentration était la plus élevée chez l'épinette noire sont l'arginine, l'acide glutmique et la proline; chez le pin gris, à part ces trois acides aminés, l'acide aspartique et la glutamine se trouvaient aussi en concentration élevées. Les concentrations en acides aminés étaient plus élevées chez l'épinette noire que chez le pin gris. Les concentrations de certains acides aminés libres peuvent s'avérer être des indicateurs plus sensibles du statut azoté des semis que la teneur en azote foliaire.

[Traduit par la revue]

Introduction

In conifers the effects of nitrogen fertilization on amino acid concentrations have been examined in relation to flowering (Barnes and Bengtson 1968), cone production (Ebell and McMullan 1970), woolly aphid infestation (Carrow and Betts 1973; Carrow 1973), seedling growth after germination (Pharis *et al.* 1964), and nitrogen metabolism in general (Durzan and Steward 1967). Baxter (1965) used amino acids for predicting the nitrogen status in fruit trees and their application to forest trees was discussed by van den Driessche (1974). The feasibility of using amino acids to predict the nitrogen status in Douglas-fir tissues was subsequently investigated by van den Driessche and Webber (1977) with encouraging results. In coniferous tree seedling nurseries, nitrogen fertilization is important for regulating seedling growth and also for inducing dormancy and frost hardiness in preparation for overwintering (Glerum 1985). Successful overwintering of containerized tree seedlings depends on adequate frost hardiness development before the seedlings are removed from the greenhouse in the fall (Colombo *et al.* 1984).

To determine the effect of fertilization on amino acid concentration and to see if amino acids can be used to indicate seedling nitrogen status, samples were collected in late fall from a jack pine (*Pinus banksiana* Lamb.) and black spruce (*Picea mariana* (Mill) B.S.P.) containerized seedling fertilization trial. Since fertilization affects both amino acid concentration and frost hardiness, it may eventually be possible to relate amino acid concentration to frost hardiness. Fertilization effects on amino acid concentrations in black spruce and jack pine seedlings, presented here, are the first step of this investigation.

Materials and methods

Ten-week-old greenhouse-grown black spruce and jack pine containerized seedlings (FH408 Japanese paper pots) were transferred in their trays from nurseries in northeastern Ontario to a greenhouse at Maple (43°51′ N latitude, 79°31′ W longitude) during the 3rd week of August 1984, where they were grown under natural light and greenhouse temperatures (20–30°C). Seedlings were fertilized to runoff weekly from September to December with Plant Products[1] 20–8–20 fertilizer at nitrogen concentrations of 0, 35, 150, or 350 ppm. A total of 64 trays (about 300 seedlings per tray) were used, i.e., eight trays per fertilizer concentration per species. Trays were located on adjacent greenhouse benches and the seedlings were not arranged in a strict experimental design because of the high degree of greenhouse environment uniformity.

In mid-November, when seedlings were 23 weeks old, 15 seedlings were randomly selected from each tray of each treatment. Growing medium was washed from the roots after which the needles were separated from the stem. Tissues were killed in liquid nitrogen and subsequently freeze-dried for 72 h. Dried needles were ground in a Wiley Mill (20 mesh) and stored at −20°C until extraction.

Amino acids were extracted from a 2-g sample in 45 mL of distilled water at 40°C for 30 min, filtered, and reextracted a second time. The combined extracts were evaporated to dryness in a rotary evaporator under a vacuum of 70 cm Hg at 50°C. The residue in the flask was redissolved with water and centrifuged at 165 000 × *g* for 25 min. The supernatant was reevaporated to about 2 mL, and this sample was brought up to 5 mL with water adjusted to pH 3 with HCl, stored in a cold room at least overnight, and recentrifuged the following day before passing the 5 mL of supernatant through a cation exchange column (Amberlite IR-120 (H^+)) with pH 3 water. Amino acids were released by passing 4 *N* NH_4OH through the column. The amino acid

[1]Mention of brand names does not constitute endorsement.

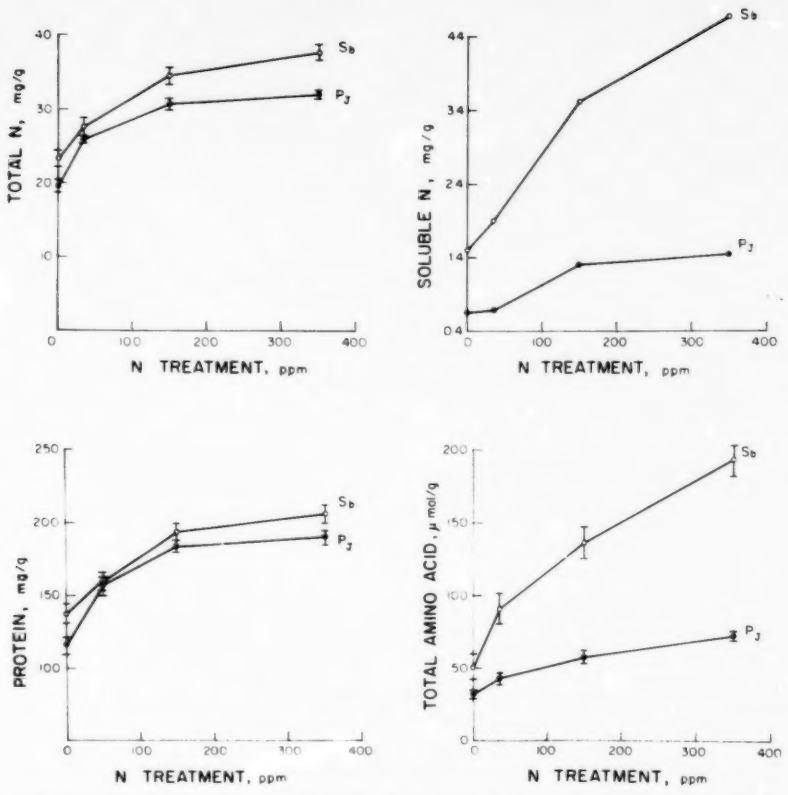

FIG. 1. Total nitrogen, soluble nitrogen, protein, and total amino acid concentrations in black spruce (S_b) and jack pine (P_j) needles as influenced by four fertilizer concentrations. Vertical bars are 95% confidence limits. No confidence limits could be calculated for soluble N because each point represents a single measurement.

eluate was evaporated to dryness and redissolved with 1 mL distilled water and 1 mL ethyl alcohol and stored in a freezer until further analyses.

The amino acids were analyzed using thin layer chromatography (TLC). The TLC plates (20 × 20 cm) were covered with a 0.15 mm thick layer of microcrystalline cellulose. The plates were spotted with 5 or 10 μL of amino acid eluate of black spruce or jack pine, respectively, and developed two dimensionally. The first solvent was 2-propanol – butanone – 1 *N* HCl (60:15:25 v/v) and the second solvent was 2-methyl-2-butanol – butanone – propanone – methanol – water – concentrated (0.88%) ammonia (50:20:10:5:15:5 v/v) as recommended by Heathcote and Haworth (1969). All amino acids, except proline, were visualized with a cadmium–ninhydrin spray. Proline was visualized with a cadmium–isatin spray.

After visualization the amino acid concentrations were quantified on the basis of spot area (Purdy and Truter 1962). Each sample and its duplicate were spotted twice and averaged.

Total amino acid concentration of the amino acid eluate was determined with the ninhydrin method and spectrophotometer at 570 μm wavelength (Siminovitch *et al.* 1964). Since proline reacts differently in this method, it was added separately as determined from the TLC plates.

Total needle nitrogen was determined from 0.3 g freeze-dried samples and the soluble nitrogen from 1-mL samples of the amino acid eluate by using the Kjeldahl method with the Büchi 320 nitrogen analyzer. Total nitrogen determinations were made in duplicate, while those of soluble nitrogen were made singly.

Protein concentrations were determined by multiplying the difference between total nitrogen and soluble nitrogen with the crude protein conversion factor of 6.25. The various nitrogen forms were subsequently subjected to an analysis of variance as regressed against nitrogen fertilizer application rate.

Results and discussion

We were able to identify only 22 free amino acids, as compared with the 24 identified by Durzan and Steward (1967) in white spruce and jack pine needles. Our procedure was specifically developed for the quantification of amino acids and of the 22 identifiable amino acids we were able to quantify 16 of them.

In both species, total N, soluble N, protein, and amino acid concentration increased markedly with increasing nitrogen fertilizer concentration (Fig. 1). Total amino acid concentrations were significantly correlated with N supply level in both species, while soluble N concentration was only significantly correlated with N supply level in black spruce (Table 1). This suggests that total amino acid and soluble nitrogen analyses may provide a better estimation of N status in coniferous needles than total N analysis.

In both species, most of the 16 amino acids increased in concentration with increasing fertilizer concentration. In sharp contrast, tryptophan concentration decreased as fertilizer concentration increased (Figs. 2 and 3).

Proline, glutamic acid, and arginine occurred in large amounts in black spruce and also showed the greatest increases in concentration with increases in fertilizer concentration. In

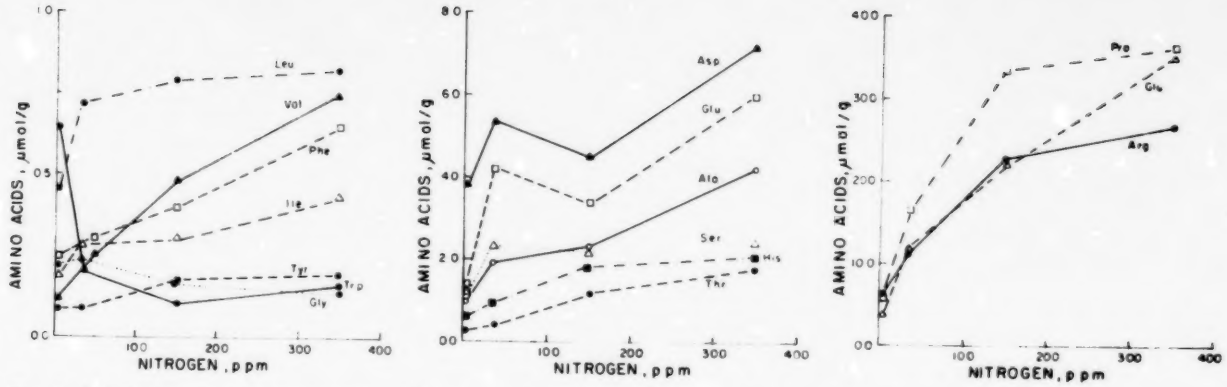

FIG. 2. Individual amino acid concentrations in black spruce needles as influenced by four fertilizer concentrations. The amino acids are grouped according to their relative concentrations and each point is the average of four observations.

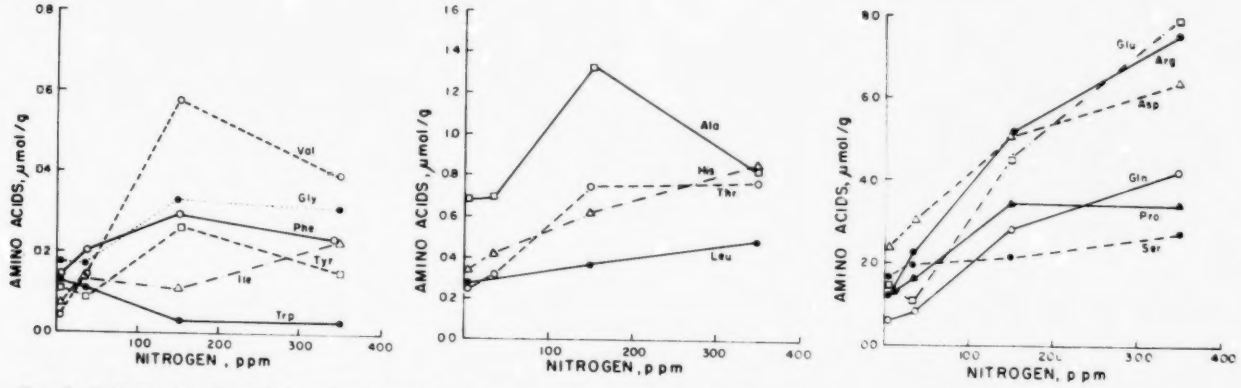

FIG. 3. Individual amino acid concentrations in jack pine needles as influenced by four fertilizer concentrations. The amino acids are grouped according to their relative concentrations and each point is the average of four observations.

TABLE 1. Coefficient of determination (R^2) and F values for regressions of nitrogen concentration in four different forms and nitrogen fertilizer application rate in the needles of black spruce and jack pine seedlings

N form	Black spruce		Jack pine	
	R^2	F	R^2	F
Total amino acids	0.95	36.13*	0.94	30.53*
Soluble nitrogen	0.92	23.91*	0.83	9.64 NS
Total nitrogen	0.86	11.89 NS	0.70	4.97 NS
Protein	0.83	9.78 NS	0.68	4.22 NS

NOTE: *, significant at the 5% level; NS, nonsignificant at the 5% level.

jack pine, these three amino acids plus aspartic acid and glutamine were most prominent.

The total amounts of soluble nitrogen and amino acids were significantly lower in jack pine than in spruce (Fig. 1), which is also reflected by the levels of the individual amino acid concentrations (Figs. 2 and 3). These observations are in agreement with those of Durzan and Steward (1967).

Arginine is generally considered to act as a source of readily available nitrogen, both because of its relative abundance and because it is the only amino acid with four nitrogen atoms in its molecule. Furthermore, arginine responds directly to nitrogen fertilization, as shown here as well as by many other investigators (Pharis *et al.* 1964; Durzan and Steward 1967; Barnes and Bengtson 1968; Ebell and McMullan 1970; Tromp 1970; Carrow and Betts 1973; Carrow 1973; van den Driessche and Webber 1977). However, proline, glutamic acid, aspartic acid, and glutamine also responded to fertilization, which is in general agreement with some other observations (Durzan and Steward 1967; Ebell and McMullan 1970; Carrow 1973). These investigators also noted that the form of nitrogen fertilizer, such as ammonia or nitrate and other nutrients, will have an effect on which of the above mentioned amino acids will show the greatest response. Furthermore, amino acids also exhibit seasonal trends or cycles (Durzan and Steward 1967; Glerum and Balatinecz 1980).

In spite of the many variables that influence amino acid concentrations there is evidence to suggest that some free amino acids may be more sensitive indicators of nitrogen status or responses to nitrogen fertilization than total foliar nitrogen. However, to make these indicator amino acids useful we have to consider all variables and calibrate the indicator amino acids accordingly.

Acknowledgements

The authors thank Ms. E. Scott for doing the nitrogen determinations and the staff of the Northern Region of our Ministry for providing us with the seedlings. They would also

like to acknowledge the many important comments of the first anonymous reviewer.

BARNES, R. L., and G. W. BENGTSON. 1968. Effects of fertilization, irrigation, and cover cropping on flowering and on nitrogen and soluble sugar composition of slash pine. For. Sci. **14**: 172–180.

BAXTER, P. 1965. A simple and rapid test, using the ninhydrin method, for the determination of the nitrogen status of fruit trees. J. Hort. Sci. **40**: 1–12.

CARROW, J. R. 1973. Free amino acids in grand fir needles and the effects of different forms of foliar-applied nitrogen. Can. J. For. Res. **3**: 465–471.

CARROW, J. R., and R. E. BETTS. 1973. Effects of different foliar-applied nitrogen fertilizer on balsam woolly aphid. Can. J. For. Res. **3**: 122–139.

COLOMBO, S. J., D. P. WEBB, and C. GLERUM. 1984. Frost hardiness testing: an operational manual for use with Extended Greenhouse Culture. Ont. Minist. Nat. Resour., For. Res. Rep. No. 110.

DURZAN, D. J., and F. C. STEWARD. 1967. The nitrogen metabolism of *Picea glauca* (Moench) Voss and *Pinus banksiana* Lamb. as influenced by mineral nutrition. Can. J. Bot. **45**: 695–710.

EBELL, L. F., and E. E. MCMULLAN. 1970. Nitrogenous substances associated with differential cone production responses of Douglas-fir to ammonium and nitrate fertilization. Can. J. Bot. **48**: 2169–2177.

GLERUM, C. 1985. Frost hardiness of coniferous seedlings: principles and applications. *In* Evaluating seedling quality: principles, procedures and predictive abilities of major tests. *Edited by* M. L. Duryea. For. Res. Lab., Oregon State Univ., Corvallis, OR. pp. 107–123.

GLERUM, C., and J. J. BALATINECZ. 1980. Formation and distribution of food reserves during autumn and their subsequent utilization in jack pine. Can. J. Bot. **58**: 40–54.

HEATHCOTE, J. G., and C. HAWORTH. 1969. An improved technique for the analysis of amino acids and related compounds on thin layers of cellulose. II. The quantitative determination of amino acids in protein hydrolysates. J. Chromatogr. **43**: 84–92.

PHARIS, R. P., R. L. BARNES, and A. W. NAYLOR. 1964. Effects of nitrogen level, calcium level and nitrogen source upon the growth and composition of *Pinus taeda* L. Physiol. Plant. **17**: 560–571.

PURDY, S. J., and E. V. TRUTER. 1962. Quantitative analysis by thin-film chromatography. Analyst (London), **87**: 802–809.

SIMINOVITCH, D., H. THERRIEN, F. GFELLER, and B. RHEAME. 1964. The quantitative estimation of frost injury and resistance in black locust, alfalfa, and wheat tissues by determination of amino acids and other ninhydrin-reacting substances released after thawing. Can. J. Bot. **42**: 637–649.

TROMP, J. 1970. Storage and mobilization of nitrogenous compounds in apple trees with special reference to arginine. *In* Physiology of tree crops. *Edited by* L. C. Luckwill and C. V. Cutting. Academic Press, London and New York. pp. 143–159.

VAN DEN DRIESSCHE, R. 1974. Prediction of mineral nutrient status of trees by foliar analysis. Bot. Rev. **40**: 347–394.

VAN DEN DRIESSCHE, R., and J. E. WEBBER. 1977. Seasonal variations in a Douglas-fir stand in total and soluble nitrogen in inner bark and root and in total and mineralizable nitrogen in soil. Can. J. For. Res. **7**: 641–647.